Synopsis for Registration as Research Student

For

Doctoral Degree

On

“Plant Nutrition Prediction Using Microscopic Imaging”

By

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Abstract

The Green revolution had taught farmers to use technology to meet the growing demand for food by using nutrient fertilizers and agrochemicals. To meet the requirements of an ever growing population, farmers will keep on adding fertilizers and agrochemicals in soil, which will result in soil pollution and soil degradation. According to the Food and Agriculture Organization of the United Nations, the degradation of one third of the world's soils has already released up-to 100 gigatonnes of carbon to the atmosphere, resulting in climate change. To avoid the exploitation of agrochemicals and nutrient fertilizers, a proper management of fertilizer application needs to be present in the farm.

The availability and balance of essential nutrients directly influence growth, yield, and overall vitality. As we delve deeper into an era of precision agriculture and sustainable practices, the demand for innovative techniques to detect and analyze plant nutrients with precision has surged.

This proposal intends to explore the microscopic view of the plant leaves to detect the effect of nutrient deficiency on the structure of stomata using image processing techniques and provide a solution to the farmers to know the nutrient content of a plant in an easy way.

1. Introduction

Nutrient analysis in plants refers to the process of assessing the concentration of various essential nutrients within plant tissues. This analysis is crucial for understanding the nutritional status of plants, diagnosing nutrient deficiencies or imbalances, and optimizing plant growth and productivity. Just like humans require a balanced diet for proper health, plants also need a variety of nutrients to thrive and achieve their full potential.

The primary nutrients required by plants are often referred to as **macronutrients**. These include:

Nitrogen (N): Essential for leaf and stem growth, nitrogen is a major component of chlorophyll, which is necessary for photosynthesis.

Phosphorus (P): Important for root development, flowering, and fruiting. It plays a key role in energy transfer and storage within the plant.

Potassium (K): Vital for overall plant health and resilience. It regulates water uptake, improves disease resistance, and enhances fruit quality.

Additionally, there are secondary nutrients and micronutrients that are equally important, albeit required in smaller quantities:

Secondary Nutrients:

Calcium (Ca): Essential for cell wall structure and root development.

Magnesium (Mg): A component of chlorophyll, crucial for photosynthesis.

Sulfur (S): Important for protein synthesis and enzyme activity.

Micronutrients (Trace Elements):

Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Molybdenum (Mo), Boron (B), and Chlorine (Cl): These micronutrients are required in very small amounts but are essential for various physiological and metabolic processes within the plant.

The process of nutrient analysis in plants involves:

Sample Collection: Plant tissues, such as leaves, stems, or roots, are collected for analysis. These samples should be representative of the plant's current nutritional status.

Sample Preparation: The collected plant tissues are cleaned, dried, and ground to a consistent texture for analysis. This ensures accurate and uniform results.

Chemical Analysis: Various laboratory techniques are used to determine the nutrient concentrations in the plant samples. Spectrophotometry, titration, and atomic absorption spectroscopy are common methods for nutrient quantification.

Data Interpretation: The nutrient analysis results are compared to established nutrient guidelines or standards to identify nutrient deficiencies or excesses. This information helps in diagnosing plant health issues and formulating appropriate solutions.

Fertilization Strategy: Based on the nutrient analysis results, farmers, gardeners, and agricultural professionals can develop precise fertilization plans. These plans ensure that plants receive the right nutrients in the right proportions to support optimal growth and productivity.

Soil Management: Nutrient analysis of plants often goes hand-in-hand with soil analysis. Soil nutrient levels influence the availability of nutrients to plants. By considering both plant and soil analyses, experts can make informed decisions about soil amendments and fertilization practices.

In summary, nutrient analysis in plants is a fundamental aspect of agriculture and horticulture. It helps ensure healthy plant growth, higher crop yields, and sustainable

agricultural practices. Proper nutrient management based on accurate analyses contributes to increased food production, improved plant quality, and environmental stewardship.

Methods Used for Plant Nutrition Detection:

Plant nutrition detection involves assessing the nutrient levels and overall health of plants to optimize their growth and productivity. There are several methods used for plant nutrition detection, ranging from traditional techniques to modern technologies. Here are some commonly used methods:

1. **Soil Testing**:

Soil is the basic foundation for agriculture which is the major source of nutrients needed by plants for growth. The major elements of soil are Nitrogen, Phosphorus and Potassium and the minor nutrients are Iron, Magnesium, Zinc and Copper. For proper development and growth of plants, these major and micro nutrients must be in the appropriate amount. To find the amount of nutrients present in the soil, soil needs to be tested. Soil testing involves analyzing the composition and nutrient content of the soil in which plants are growing. This helps determine the availability of essential nutrients and guides the application of fertilizers. Common soil tests include measuring pH, nutrient content (N, P, K, etc.), organic matter, and cation exchange capacity. Soil testing is a crucial method for assessing the nutrient composition and overall health of the soil in which plants are grown.

Researchers are working on developing an efficient, portable and less time consuming way to test soil directly on the field. On studying the literature of soil testing methods, it has been found that a lot of research is going on in this area in other countries but India is still lacking behind. **Decision systems** are formed based on the known soil samples in Dushu village, China [1], where a particular field study was undertaken and a model was created to provide suggestions for fertilization. In another research study it was found that with the use of **Soil Test Kit (STK) and Rapid Soil Testing (RST)** of the Bureau of Soils and Water Management, the soil sample is mixed with different chemicals for different soil nutrients. The chemical reaction develops a change in color and also the intensity of color is varied depending on soil nutrient content. This change

in color is detected with the help of image processing and the amount of soil contents are determined [2].

Vision system has also been developed for soil nutrient analysis using fuzzy logic [3]. Application of **IOT** in soil management has been carried out by checking the landscape water [4]. Few researchers are also working on automated control systems for fertilizer application based on soil content detection [5]. One of the researchers has used the **RGBC color sensor** base voltage divider circuit for determination of soil nutrients [6].

Various methods are developed for soil health monitoring but still the laboratory method is the most widely used method because of its accuracy. But the laboratory method is time consuming and the number of laboratories is also not sufficient for a country like India where around 70% of people's livelihood is on agriculture.

2. Leaf Tissue Analysis:

This method involves analyzing the nutrient content of plant leaves. By sampling and analyzing the leaves, you can determine which nutrients are deficient or excessive in the plant. This allows for targeted fertilization. Nutrient deficiencies often manifest as characteristic symptoms on leaves, helping identify specific issues.

The process of leaf tissue analysis for nutrient content consists of selecting proper leaves, preparing them to remove contaminants, drying to remove moisture content, grinding and use of suitable extraction solution to extract nutrient content. This is one more laboratory method which is time consuming.

3. Plant Sap Analysis:

Plant sap is a watery fluid of plants. Plant sap analysis measures the nutrient content in the sap of plants. This method is used to diagnose nutrient imbalances and monitor plant health. It's especially useful for rapidly identifying short-term nutrient deficiencies. In the plant sap analysis method, the sample can be the watery fluid from the stem or mature and healthy leaves. There are different methods to extract sap from plant tissues. Some common techniques include using a pressure chamber or a specialized tool that applies a slight vacuum to the leaf to encourage sap flow. The

extracted sap is then collected in a container. With the help of portable tools as shown in Fig.1.1, the amount of nutrients can be determined.

Fig.1 Plant Sap Analyser for pH analysis

Plant sap analysis is particularly useful for crops with rapid nutrient uptake fluctuations, crops grown in controlled environments (greenhouses, hydroponics), and situations where traditional soil testing may not provide timely information. It also enables fine-tuning of fertilization strategies in real time. The specific procedures and methodologies for plant sap analysis can vary based on the equipment available, the plant species being tested, and the laboratory conducting the analysis. It's advisable to work with experienced professionals or laboratories that specialize in plant sap analysis for accurate and meaningful results.

4. Visual Symptoms:

Nutrient deficiencies and toxicities often manifest as specific visual symptoms on plant leaves, stems, and fruits. These symptoms, such as yellowing leaves (chlorosis), necrosis, stunted growth, and color changes, can help diagnose nutrient imbalances. Various researchers have utilized this method and implemented image processing techniques to diagnose plant diseases.

Different nutrition deficiencies leave a different symptom on the plant appearance and structure. By having a visual of the effect of nutrition deficiency, one can predict the amount of nutrients present in the plant. This method is online and less time consuming but needs a strong database for comparison purposes. Also the prediction can only be made once there is complete nutrition deficiency and because of which it affects the plant growth and shows some symptoms.

5. Electrical Conductivity (EC) and pH Measurement:

Electrical conductivity measures the ability of a solution (usually soil or nutrient solution) to conduct an electrical current. It indirectly reflects the concentration of dissolved ions, including nutrients. pH measurement is crucial because it affects nutrient availability in the soil and uptake by plants.

6. Remote Sensing:

Modern technologies such as drones equipped with multispectral or hyperspectral cameras can capture images of crops. Hyperspectral imaging is an advanced remote sensing technique that involves capturing and analyzing the electromagnetic spectrum at a high level of spectral detail. These images can be used to detect nutrient deficiencies, stress, and other plant health issues by analyzing the reflectance patterns of different wavelengths.

Hyperspectral imaging collects data across a broad range of wavelengths, typically covering the visible, near-infrared, and sometimes infrared parts of the electromagnetic spectrum. Unlike standard color cameras that capture three bands (red, green, and blue), hyperspectral cameras capture hundreds of narrow and contiguous bands, allowing for highly detailed spectral information.

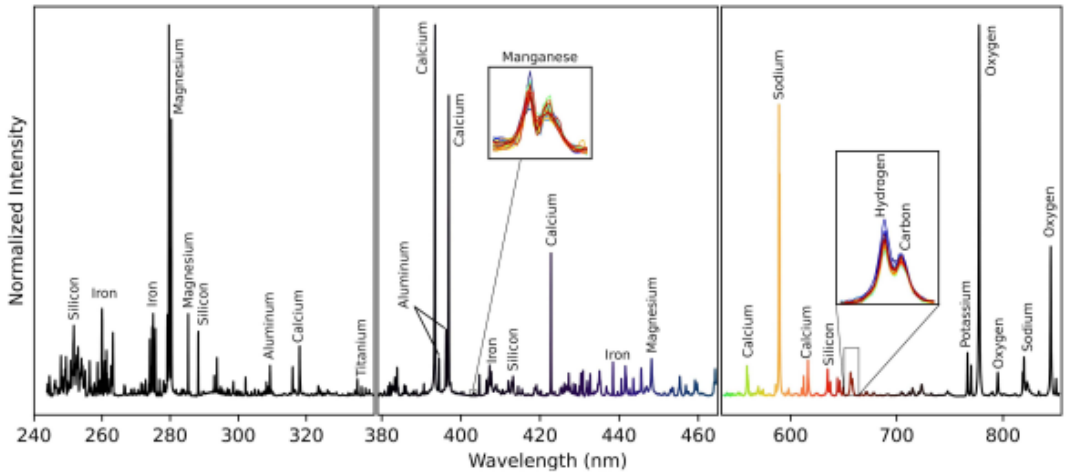

Fig.2 Hyperspectral Imaging

Hyperspectral imaging technology is continuously evolving, and its applications in agriculture are expanding. However, it requires specialized equipment which has a high cost and will not be a choice of small scale farmers.

7. Chlorophyll Meters:

Chlorophyll meters measure the chlorophyll content in leaves. Since chlorophyll is essential for photosynthesis, its levels can indicate the plant's health and photosynthetic activity, which in turn are influenced by nutrient availability.

Fig.3 Chlorophyll Meter

A chlorophyll meter, also known as a SPAD meter (Soil Plant Analysis Development), is a handheld device used to measure the relative chlorophyll content in plant leaves. Chlorophyll is a vital pigment responsible for photosynthesis, the process by which plants convert light energy into chemical energy to fuel their growth and development. Monitoring chlorophyll levels can provide insights into plant health, photosynthetic activity, and nutrient status.

A chlorophyll meter measures the transmittance of light through a leaf, particularly in the red and infrared regions of the electromagnetic spectrum. The device emits both red and infrared light onto the leaf surface. Chlorophyll strongly absorbs red light for photosynthesis, while the cell structure reflects infrared light. By comparing the transmittance of these two wavelengths, the chlorophyll meter calculates the chlorophyll content in the leaf.

Chlorophyll meters are user-friendly and provide rapid results. They are particularly useful for on-the-spot assessments in the field. However, like any tool, their accuracy depends on proper usage, calibration, and understanding of the underlying principles. Chlorophyll meters provide a relative measurement of chlorophyll content and do not directly quantify absolute chlorophyll concentrations. Other factors, such as leaf

thickness and structural variations, can affect meter readings. Interpretation of chlorophyll readings requires experience and consideration of other factors like plant species, leaf age, and environmental conditions.

8. Isotope Techniques:

Isotope labeling involves introducing stable isotopes of nutrients into the soil or nutrient solution. The isotopic tracer technique is a powerful method used in plant research to study various aspects of plant physiology, nutrient uptake, and metabolic pathways. It involves introducing stable or radioactive isotopes into plants and tracking their movement, incorporation, and transformation within the plant's biological processes. This technique provides valuable insights into plant nutrient utilization, root activity, water uptake, and other physiological processes.

The isotopic tracer technique offers a non-destructive and highly informative way to investigate complex plant processes. However, it requires specialized equipment, expertise in isotope analysis, and a deep understanding of plant physiology and biochemistry.

The choice of method depends on factors such as the plant type, growing conditions, available resources, and the level of precision required. Often, a combination of methods is used to comprehensively assess plant nutrition and health.

The various other methods as we discussed in the first chapter makes us understand that there are several ways to detect nutritional status of plants but none of them predict the nutritional deficiencies at an early stage, so as to avoid major nutrient loss and less yield. Looking into the problem, it has been observed that an online system that can be used to detect the plant nutritional status and can suggest the proper nutritional uptakes is required.

The proposed method will be able to monitor the nutritional status online with the help of microscopic images which actually checks the effect of different nutrients on the cellular structure of the plant leaves.

2. Motivation

Presently to get soil tested, the best method is laboratory method. The only disadvantage of laboratory methods is that it is highly time consuming. These tests

are actually performed in the laboratory situated at fixed locations and the farmer needs to visit the laboratory so as to get the soil tested. These tests need mixing of chemicals manually and take a long time to show results because of manual intervention. This in turn increases the waiting time and efforts of farmers. Also, for proper analysis of soil manure and fertilizers that needs to be added in the soil depending on soil test, farmers need to visit other departments. The laboratories are also limited in number and mostly located in cities. Because of these reasons, farmers intend not to visit the labs to get soil tested. This in turn affects the growth of crops as the proper amount of manure is not analyzed.

The development of an automated system will help farmers detect and identify plant nutrition and improve the quality of crops.

3. Literature Review

M. Mudholkar, P. Mudholkar, V. H. R. Dornadula, K. Sreenivasulu, K. Joshi and B. Pant's establishment of a program for monitoring plant health is covered in this study, which will evaluate certain environmental factors that impact plants, such as temperature, humidity, and light intensity. Restore soil moisture as well. The Arduino Uno development boards transmit all of this data to the Ubidot IoT (Iot) cloud platform. If there is a discrepancy between the number of sensor nodes in the data and the actual number, the user's smartphone will be notified.[7]

"Real-Time Robotic Vision Application for Health Monitoring of Paddy Plants: was designed by G. Bej et al. The proposed work estimates the chlorophyll content of the plant while navigating the robotic vehicle in the field. An image acquisition arrangement has been mounted facing the ground on the robotic vehicle which captures on-field images. Color information from these images is compared with the digitized reference leaf color chart. [8]

A compact optical sensor based on photometric detection of soil nutrients is developed. The sensor consists of three light emitting diodes (LEDs) and a simple data I / O circuit using a PIC (peripheral interface controller) device. The wavelength of LEDs is chosen to fit the absorption band of chemical reagents whose colour develops by reaction with soil nutrients. The sensor is applied for detection of six soil nutrients: ammonia nitrogen (NH 4 –N), nitrate nitrogen (NO 3 –N), available phosphorus (P 2 O 5), available iron (Fe), exchangeable manganese (Mn) and exchangeable calcium (CaO) from colour changes caused by addition of chemical

reagent in a transparent plastic cell. The results are compared with those calculated using absorbance of solutions measured by spectrophotometer. [9]

“One shot evaluation of NPK in soils by “electronic tongue” research by Maria Khaydukova, Dmitry Kirsanov, Subrata Sarkar, Subhankar Mukherjee, Julia Ashina, Nabarun Bhattacharyya, Somdeb Chanda, Rajib Bandyopadhyay, Andrey Legin uses a potentiometric multisensor system was used for simultaneous determination of the N, P and K in the water extracts of the soils. It was shown that potentiometric sensors with multivariate data processing can be used for assessment of all three macronutrients, along with estimation of pH and conductivity of soil samples. [10]

Fusion of visible and near infrared images are used to calculate the Normalized Difference Vegetative Index (NDVI) to measure and monitor plant health. A robot is designed and implemented to provide field based analysis of plant health using visible and near infrared spectroscopy.[11]

Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum were studied.[12]

“Analysis of Leaf Parameters Measurement of Cucumber Based on Image Processing” paper discusses image processing carried on measuring cucumber leaf parameters. Leaf area of cucumber is determined with image processing, which results are compared with the results of grid counting, paper weighing and leaf area meter method. Leaf maximum length and leaf maximum width of cucumber are determined with image processing, which results are compared with the results of grid counting and leaf area meter method. The experimental result shows that image processing for measuring cucumber leaf parameters is high accuracy and precision, multi-parameter, strong practicability, advanced technology, and the measurement can be done without breakage, which provides the theory basis for developing PDA leaf area.[14]

Using microscopic images to predict plant diseases in a deep learning environment, a proactive plant disease prediction system is proposed which uses deep learning models Convolution Neural Networks. Using CNN makes the system more robust, unlike variations observed using machine learning algorithms.

By using microscopic images, pathogenic activity which starts much before the disease fully develops can be analyzed for predicting disease.[15]

REVIEW PAPER-"A balancing act: how plants integrate nitrogen and water signals", by Viviana Araus has shown how combinatorial transcriptional responses to N and W status might drive crop phenotypes.[16]

3.1 Identified Open Research Problem

After carrying out a literature survey, the following research problems have been identified.

1.Application of microscopic imaging in nutrition management.

2. Need nutrition prediction well before disease occurs.

4. Methodology

Microscopic imaging, also known as microscopy, involves the use of specialized instruments to magnify and visualize objects that are otherwise too small to be seen with the naked eye. Microscopic imaging has a wide range of applications across various scientific fields, including biology, medicine, materials science, and more.

To investigate the nutrient contents in the plant, microscopic images of plant leaves will be taken such that the structure of the leaf stomata is visible.

Leaf Anatomy:

Leaf anatomy refers to the internal structure of a leaf, which is responsible for its various functions such as photosynthesis, transpiration, and gas exchange. The structure of a leaf is adapted to maximize its efficiency in performing these tasks. Here's a breakdown of the key anatomical features of a typical leaf:

- **Cuticle:** The outermost layer of the leaf is called the cuticle. It's a waxy, waterproof layer that helps reduce water loss through evaporation and also protects the leaf from damage.
- **Upper Epidermis:** This is a single layer of transparent cells that covers the upper surface of the leaf. It provides protection and allows light to enter the leaf for photosynthesis.
- **Palisade Mesophyll:** Located just beneath the upper epidermis, this layer consists of tightly packed, vertically oriented cells called palisade cells. These cells contain a high concentration of chloroplasts, which are responsible for photosynthesis.

- **Spongy Mesophyll:** Beneath the palisade mesophyll, there's a layer of loosely arranged cells known as spongy mesophyll. These cells also contain chloroplasts and facilitate gas exchange and some photosynthesis. The air spaces between these cells allow for the diffusion of gases like carbon dioxide and oxygen.
- **Lower Epidermis:** Similar to the upper epidermis, the lower epidermis is a protective layer of cells on the lower surface of the leaf. It contains specialized structures called stomata.
- **Stomata:** These are small openings or pores found on the lower epidermis of the leaf. Each stoma is surrounded by two specialized cells called guard cells. Stomata play a crucial role in regulating gas exchange and transpiration. They allow carbon dioxide to enter for photosynthesis while enabling the release of oxygen and excess water vapor.
- **Guard Cells:** These are specialized cells that flank each stoma. They can change shape to open or close the stoma, controlling the movement of gases and water vapor in and out of the leaf.
- **Vascular Bundles (Veins):** These are the vascular tissues that run through the leaf, primarily consisting of xylem and phloem. Xylem transports water and minerals from the roots to the leaves, while phloem carries the products of photosynthesis (such as sugars) from the leaves to other parts of the plant.

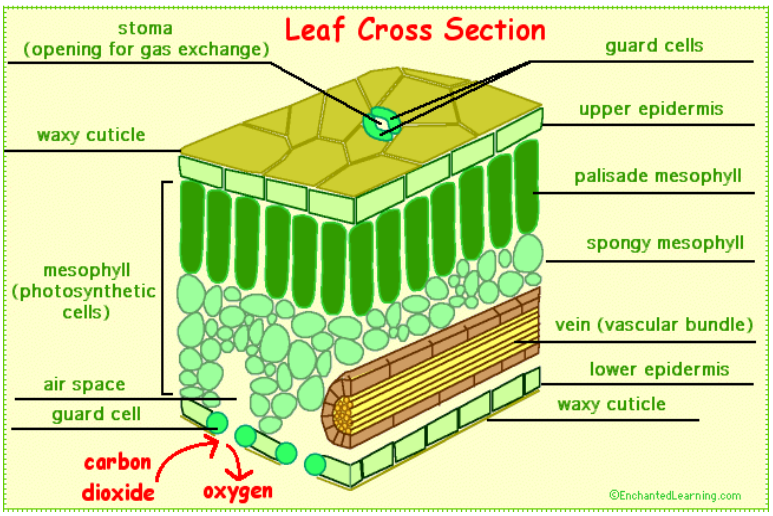

Fig. 4 Leaf Anatomy

Effect of Nutrient Deficiency on Stomatal Structure:

Botanical studies have shown that major plant nutrients nitrogen, potassium and phosphorus have significant effects on stomatal structure.

1. **Effect of nitrogen deficiency**:

   Soil nitrogen deficiency can have several effects on plant leaf tissue. Nitrogen is an essential nutrient for plants and plays a crucial role in various biological processes like chlorosis, plant growth, flowering, photosynthesis and susceptibility to diseases. When plants experience nitrogen deficiency, it can lead to changes in leaf morphology and cellular structure. Effects of nitrogen deficiency on leaf cell structure are

- Reduced cell size
- Decreased cell number
- Altered cell shape

2. **Effect of potassium deficiency:**

   Potassium plays an important role in opening and closing of stomata. This stomatal activity is important for plant transpiration. Potassium (K) deficiency in plants can lead to various observable changes in leaf cell structure.

- Reduced cell size
- Thin cell walls
- Chloroplast Abnormalities

3. **Effect of phosphorus deficiency:**

   Phosphorus increases the content and quality of proteins essential for stomatal activity. Deficiency of phosphorus leads to

- Reduced Stomatal Opening
- Water Stress

The proposed methodology utilises this effect of nutrients on stomatal structure using image processing.

The overview of the proposed methodology is shown in Fig 6.

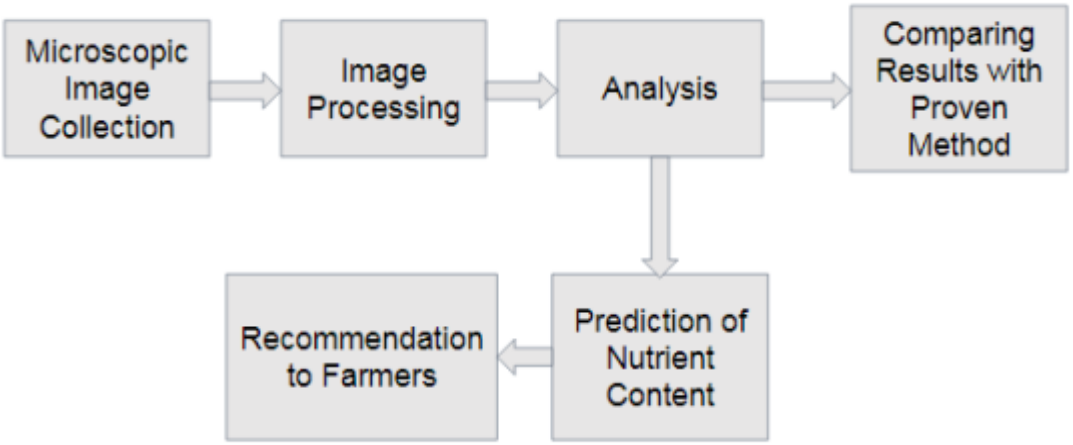

Fig. 6 Block diagram of the proposed methodology

Microscopic images of plant leaves covering stomata will be collected for healthy and unhealthy plants. Image processing of the obtained images will be done using python to reduce the software cost and will give an analysis of the stomatal structure. The results will be compared with the proven laboratory methods for validity. A prediction system based on the analysis of images can be done using machine learning algorithms.

5. Conclusion

This research will utilize these effects of nutrient deficiency on stomatal structure and address the following points that are currently lacking in this domain.

1. Developing a portable device which can be carried in the farm for plant nutrient detection.
2. Developing a database of microscopic images for different nutrient content.
3. Developing a proper fertilizer suggestion system based on nutrient content.

The results of this proposal will be helpful for farmers to analyze the nutrient status of his crops at his own place and in turn the amount of fertilizer and manure required for the soil for effective growth of crops. A proper and healthy crop will not only encash proper returns to the farmer but also a healthy society.

6. Proposed Work Plan

Table 1: Year-wise research work plan

Activities	Year		
	(2023-24)	(2024-25)	(2026-27)
Literature Survey			
Understanding Tools for Problem Solving			
Implementation of Imaging System			
Data Collection			
Image Processing			
Elaborating A Methodology for Prediction			
Comparative Analysis of Results			
Writing Research Articles			
Submitting Research Articles to Professional Journals.			
Pre- Ph.D. Synopsis Submission			
Thesis Writing, Thesis Submission			

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